SOLID-STATE, ^{13}C, CROSS-POLARIZATION, "MAGIC-ANGLE" SPINNING, NMR SPECTROSCOPY STUDIES OF SEWAGE SLUDGE

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ABSTRACT

Using ^{13}C, solid-state NMR, we examined sludges and composts obtained from varied locations. A minimum of sample preparation is required with this technique. The samples are simply dried and ground before they are packed into the rotor. The spectra of the sewage sludges examined are composed of four broad envelopes of chemical shifts representative of carbonyl carbon, aromatic carbon, aliphatic carbon adjacent to –OH or –N, and aliphatic carbon.

The intensities of the carbonyl carbon, aliphatic carbon adjacent to –OH and –N and aliphatic carbon resonances were less in the compost spectra, when compared with the intensity of the aromatic carbon resonances, than in the sewage sludge spectra. Spectra of four reference compounds (stearic acid, cellulose, lignin, and protein (keratin)) are included to assist in the interpretation of the sample spectra. Interrupted decoupling experiments were conducted to simplify these complex spectra and estimate the amount of nonprotonated (branched and carbonyl) carbon they contain. This preliminary solid-state, NMR study of sludges demonstrates that this technique is an effective tool for studying these complex systems.

INTRODUCTION

The processes of sewage sludge production, decomposition, and stabilization produce products that are chemically complex and difficult to fractionate (Wershaw 1981; Hartenstein 1981). Extraction procedures that have a minimal effect on the chemical integrity of these samples extract only a small portion of the carbon present. Procedures that extract more significant amounts of carbon affect covalent bonds, altering the chemical nature of the sample (Worobey and Barrie Webster 1981). To profile the total organic content of sludges and their decomposition products, without altering their chemical nature, one must use a method that can be applied to the intact sample.

Studies have been conducted using cross-polarization, magic-angle spinning, ^{13}C solid-state NMR (CP-MAS NMR) to study such diverse systems as polymers, sediments, coal, cellulose, lignin, and wheat (Earl and Vander Hart 1979; Hatcher et al. 1980a,b; Wemmer et al. 1981;

Bartuska et al. 1980; Schaefer et al. 1981; Earl and VanderHart 1980; Baianu and Forester 1980; Schofield and Vianu 1982). To obtain high resolution ^{13}C spectra in the solid state, the problems associated with intimate lattice interactions must be overcome. These interactions result in extremely broad resonance lines due to homonuclear and heteronuclear dipole coupling. The former can be eliminated by working with a magnetically dilute system, such as ^{13}C, and the latter by the use of very high-powered, decoupling at the resonance frequency of the heteronucleus (Pines et al. 1973; Mehring 1976). A second experimental problem is the very long relaxation times (T_1s) associated with magnetically dilute nuclei and the poor signal-to-noise ratio that is a practical experimental result. This problem is overcome by the use of a magnetization transfer from the abundant proton spins to the dilute ^{13}C nuclei. This cross-polarization technique results in vastly shorter times for good signal-to-noise ratios (Pines et al. 1973; Hartman and Hahn 1962). The final problem arises because the position of a given resonance line is a function of the exact orientation of the molecule relative to the magnetic field (chemical shift anisotropy). In amorphous samples this implies

that the resonance line will be anisotropically broadened to a width of about 1 to 2 kHz for ^{13}C. To narrow the chemical shift anisotropy, the sample is spun at approximately 2 kHz about the "magic angle," which is 54.7° from the applied field direction (Andrew 1981), where the "magic angle" arises because the shift anisotropy contains a $(1 - 3 \cos^2 \theta)$ dependence and is equal to zero when $\theta = 54.7°$. The resulting high resolution spectra contain lines that can correspond to physical as well as chemical differences in structure within the solid. An in-depth explanation of the CP-MAS NMR technique may be found in Wemmer et al. (1981), Schaefer et al. (1977), and Reinhold (1982).

In this preliminary study, we apply CP-MAS NMR to sewage sludges to determine what information can be obtained about sludge organic matter using this technique.

EXPERIMENTAL

The samples studied represent a wide variety of sample types. Sludges were collected from four municipal waste treatment facilities with sewage input consisting of residential and industrial wastes. These sludges, designated S1, S2, S3, and S4, were field-stored for several years while field studies were being conducted. Two composts, designated C1 and C2, were prepared from sludges by the Beltsville method (Epstein et al. 1976). Compost C2 was prepared from sludge S3. An anaerobic sludge, designated AS1, produced from tannery wastes in a bench scale anaerobic reactor, was also examined. All samples were lyophilized to remove free water. The water content of the sewage sludge and compost samples ranged from 2 to 43% water before drying. The dried samples had the appearance of organic-rich soils.

Most of the water solubles in the sewage sludges and composts were probably lost during processing, when the solids were concentrated, or during storage, by leaching. The anaerobic tannery sludge was sampled by taking an aliquot of the digestor suspension that contained about 6% solids. Therefore, the lyophilized sample of the anaerobic sludge probably contains water-soluble components that were lost from the sludges and composts. The only other treatment required to prepare the samples for analysis was grinding to provide a homogeneous mixture with a particle size that could be easily packed into a rotor. These samples were ball-milled in 6-L jars for 96 h (Polcin and Bezuch 1978). This exhaustive grinding procedure was employed because these samples were used in other studies that required a small particle size. A minimum of grinding is required to prepare the samples for NMR analysis. Solid-state NMR can detect changes in the degree of order (crystallinity) in samples that can result from extensive ball-milling (Kolodeiejski et al. 1982). These sludges and composts may contain domains of order (cellulose, protein, etc.) that could be affected by the grinding method employed.

The organic content of the sewage sludge and compost samples was estimated to range from 25 to 49% for the sludges and 19 to 37% for the composts based on weight loss of the lyophilized samples after heating in a muffle furnace at 450°C for 16 h.

The organic component of sewage sludges is composed of the organics in the sewage that were not metabolized by the process microorganisms and the metabolites and cell components of these microorganisms. Four reference compounds were selected, which were thought to be representative of the carbon types found in these samples.

Stearic acid was chosen to represent the aliphatic carbon and a portion of the carbonyl carbon. Cellulose, in the form of wood pulp, was selected to represent the carbohydrate carbon, because it is more resistant to microbial breakdown than other carbohydrates and is the most ubiquitous carbohydrate found in nature. Lignin, which is especially resistant to microbial action, was selected to represent the aromatic carbon of sludge. NaOH extracts of sludge contain an aromatic component that some researchers believe is partially derived from lignin (Hartenstein 1981). Finally keratin, hair protein, another organic resistant to microbial decomposition, was selected to represent protein carbons. Spectra of the reference compounds were obtained to determine how the various carbon types they represent respond to the instrumental parameters used in this study.

Spectra were obtained on a JEOL FX 60 Q-S NMR spectrometer operating at a ^{13}C frequency of 15 MHz. A spectral width of 8000 Hz was selected to minimize acquisition time. The ^{1}H decoupling r.f. irradiation field strength was 11 G. Chemical shifts were determined with respect to tetramethylsilane using hexamethylbenzene (HMB) as a secondary reference assigning a shift of 17.36 ppm to the aliphatic peak. Spectra were obtained using a contact time of 1 ms. The

composts contained the least organic carbon and required 50 000 scans to obtain a marginally acceptable signal-to-noise ratio. The compost sample, which contained 19% organic, approaches the minimum limit of organic that will provide an acceptable signal-to-noise ratio for these samples using what we consider to be a reasonable number of scans (5×10^4). Barron et al. (1980) obtained a spectrum of a soil that contained 6% organic by taking 2×10^5 scans on a CXP 100 spectrometer operating at 22.63 MHz. The differences between these samples may not be due only to instrumentation but may also be due to the differences in the amount of paramagnetic species these samples contain. We are currently studying the effects iron has on the solid-state spectra of some of the components in complex systems, such as sludges. All sludge and compost spectra are the result of 50 000 scans, which took 20.8 h to acquire, and all reference compound spectra are the result of 3000 scans, which took 1.2 h to acquire. The samples were spun at 2.1 kHz to minimize side bands. Care was taken to maintain the proper spin rate, because, in our system, the angle of the rotor changes with the rate of spin. When the magic angle setting is being determined, using HMB, the intensity of the downfield peaks (aromatic and carbonyl) is affected greatly by slight changes in the rotor angle, because these groups have large chemical shift anisotropies.

The solid-state ^{13}C CP-MAS NMR spectra of sewage sludges are complex. A useful technique for simplifying complicated spectra and for assigning the resonance peaks of nonprotonated carbons has been described by Opella and Frey (1979). This interrupted decoupling technique relies on the fact that, in the absence of proton decoupling, the proton-carbon dipolar interaction is a powerful relaxation mechanism. This proton-carbon interaction has a $1/r^3$ dependence and consequently is much stronger for carbons with directly bonded protons (~1 Å separation) than for carbons that have only nearest neighbor protons ($\geq$2 Å separation). By inserting a 50-μs delay with no decoupling between the cross-polarization pulse (during which the carbon magnetization is generated) and the start of data acquisition, one can greatly enhance the relaxation of the protonated carbons over that of the nonprotonated carbons. The practical result of this particular pulse sequence is that the reso-

nance signals of protonated carbons are vastly attenuated relative to nonprotonated carbons. This process causes a somewhat reduced signal-to-noise ratio for all signals. (One aspect of this pulse sequence is that methyl carbon resonances also remain in the spectrum, because the methyl rotation motionally reduces the proton-carbon dipole interaction for the directly bonded methyl protons. In practice this is not a serious limitation, for methyl carbons can be easily recognized by their chemical shift.)

DISCUSSION

The spectra of the four reference compounds shown in Fig. 1 illustrate the type of information ^{13}C solid-state NMR can provide. The spectrum of stearic acid (A) is composed of four peaks. The peak at 182 ppm represents the carbonyl carbon, and the sharpness of the peak is indicative of its homogeneous nature. The three peaks at 14 to 36 ppm represent the aliphatic
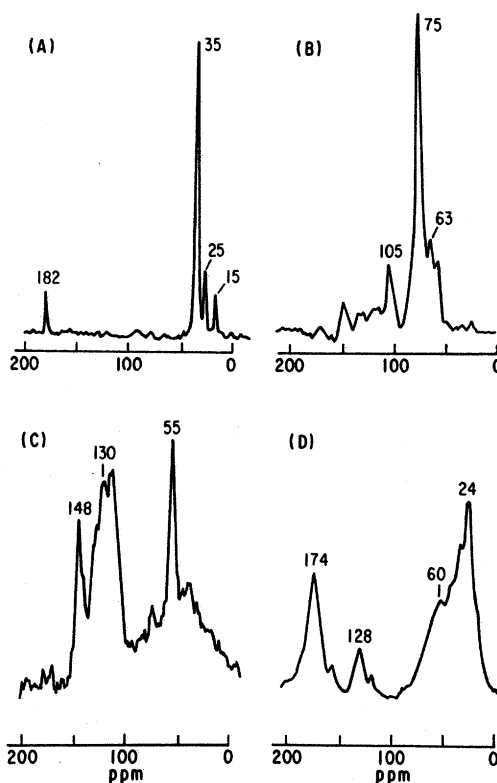

FIG. 1. CP-MAS ^{13}C NMR spectra of reference compounds (A) stearic acid, (B) wood pulp (cellulose), (C) lignin, and (D) protein (keratin).

carbons. The peak at 15 ppm is the terminal methyl, the one at 25 ppm is the methylene adjacent to the terminal carbon, and the peak at 35 ppm represents the rest of the methylenes.

Spectrum B is of wood pulp, the main components of which are celluloses and a small amount of lignin. The peak at 105 ppm is assigned to the anomeric carbon, with the C-2, C-3, and C-5 carbons at 70 to 80 ppm. The resonance at 63 ppm is assignable to the C-6 carbon. Hemicellulose acetate methyl and carbonyl peaks contribute to the 21- and 174-ppm peaks. The carbonyls of lignin also contribute to the 174-ppm peak and the lignin aromatic carbons contribute to the resonances between 128 to 150 ppm. Large molecular weight polymers, such as cellulose, which are composed of a single repeating unit, give well-resolved spectra, for they contain a limited number of carbon types (Earl and VanderHart 1980; Kolodeiejski et al. 1982).

The lignin spectrum (C), as previously shown (Bartuska et al. 1980; Schaefer et al. 1981), is that of an aromatic polymer with chemical shift dispersions from 100 to 142 ppm for the aromatic component. The two sharp resonances represent the phenyl methoxy carbons at 55 ppm and the methoxy-substituted or hydroxy-substituted phenyl carbons at 148 ppm. The aromatic component of lignin, centered around 130 ppm, is complex and produces a broad envelope composed of many aromatic carbon types.

The protein spectrum (D) contains broad peaks and lacks the resolution seen in the other reference spectra. This lack of resolution is due to the chemical heterogeneity of the protein, which produces a broad distribution of isotropic chemical shifts (Baianu and Forester 1980). This complex protein spectrum is composed of a broad envelope of carbonyl carbons centered at 174 ppm; an envelope of aromatic carbons at 128 ppm; a shoulder at 60 ppm, due to carbons adjacent to nitrogen; and a broad envelope of aliphatic carbons at 24 ppm.

These spectra represent a progression from a very simple system to a very complex one. ^{13}C CP-MAS is a relatively high-resolution technique and can resolve specific carbons in simple organic compounds or mixtures. As the systems being studied become more complex with regard to the number of different carbons present, however, the peaks become unresolvable envelopes of similar carbons. As the dispersity of carbon

types in a sample increases, the signal intensity for any one carbon type decreases. This dilution effect results in a degradation of the signal-to-noise ratio. This can be seen when spectrum (A), stearic acid, and (D), protein, are compared.

The NMR spectra of the complex systems, presented in this study (Figs. 2 to 4) provide general information with respect to four carbon types. The area between 160 and 180 ppm is representative of carbonyl carbons. These can be free acids, esters, salts, aldehydes, and ketones. The region between 110 and 140 ppm represents aromatic carbons, and shifts of 40 to 80 ppm are indicative of aliphatic carbons attached to -OH or -N, and the region 10 to 40 ppm is of nonsubstituted aliphatic carbons.

These chemical shift data are useful in interpreting the spectra of sludges in Fig. 2. Because the spectra were quite noisy, they were reproduced using 80-Hz line broadening. All four carbon types are present in the sludge spectra. There are differences in the relative amounts of each carbon type in the various samples studied. Aliphatic peaks are prominent in all samples.
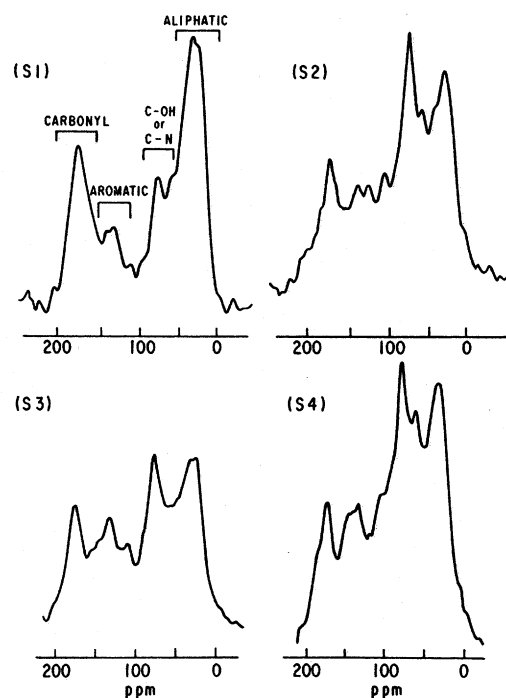

FIG. 2. CP-MAS ^{13}C NMR spectra of sewage sludges S1, S2, S3, and S4 from four municipal treatment plants.

There is a decrease in the amount of C-OH or C-N carbon and aromatic carbon relative to the aliphatic peak in sample S1, compared with the other spectra. The spectrum of sample S1 looks similar to the spectrum of protein in Fig. 1. These spectra show that differences between sludges from varied sources can be readily observed using solid-state NMR.

Composts are sludges that have been treated to maximize the decomposition of the organic components by oxidation and the metabolism of microorganisms. The spectra of two composts in Fig. 3 illustrate the effects of this additional treatment on the carbon composition. When compared with the sludge spectrum S3, it can be seen that the intensities of the aliphatic carbon and C-OH or C-N carbon regions have been reduced significantly with respect to the aromatic region. There is also a reduction in the carbonyl content. These compost spectra display almost equal amounts of all carbon types.

Spectrum AS1 in Fig. 4, which was reproduced using 10-Hz line broadening, is that of an anaerobic tannery waste sludge as it comes from the reactor with no additional aging or treatment. A considerable amount of the effluent was sampled along with the solids when this sample was collected. The spectrum of this sample is very different from the other sludge or compost spectra. The sharp resonances compare closely to those of the stearic acid spectrum (A). In fact, the major carbon components of this sample are fatty acids and a smaller amount of aromatic acids. The acids were isolated from the sample using micro methods developed by Schwartz (1974, 1977). As the tannery sludge had a pH of 7.1 before the sample was lyophilized, the acids were in the salt form. The sample was acidified on the chromatography column to liberate the free acids before derivatization. The methyl derivatives were separated and identified using capillary GC-MS and found to be predominantly C_{14}, C_{16}, and C_{18} fatty acids. There were both saturated and unsaturated forms. This tannery waste sludge was the only sample studied that was found to contain measurable amounts of these free fatty acids. Benzoic acid, phenylacetic acid, and phenylpropionic acid were also identified. The shift at 129.6 ppm is assigned to the phenyl carbons of these aromatic acids. Based on the spectrum AS1, the fatty acids appear to be the major carbon source in this sample.
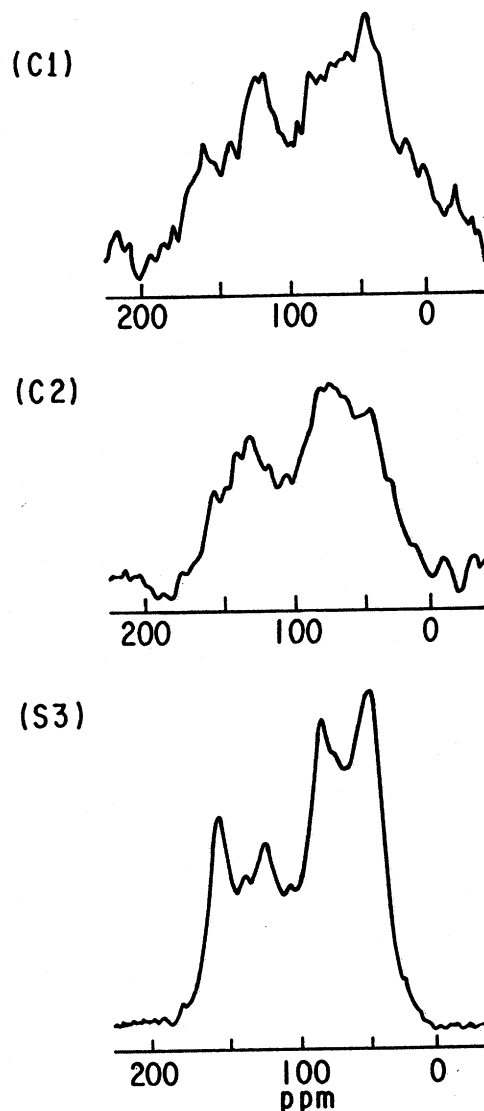
A computer program that could add the spec-

FIG. 3. CP-MAS ^{13}C NMR spectra of two composts C1 and C2 compared to sewage sludge S3. Compost C2 was prepared from sewage sludge S3.

tra of the four reference compounds in different proportions was employed to determine how closely the reference compounds approximate the carbon types found in the spectrum of sludge sample S3. The four reference spectra used were reproduced using 80-Hz line broadening. A combination of 25% stearic acid, 10% lignin, 30% protein as keratin, and 35% wood pulp was used for comparison. As seen in Fig. 5, the simulated spectrum (A) contains the major peaks found in the sludge spectrum S3. There are differences
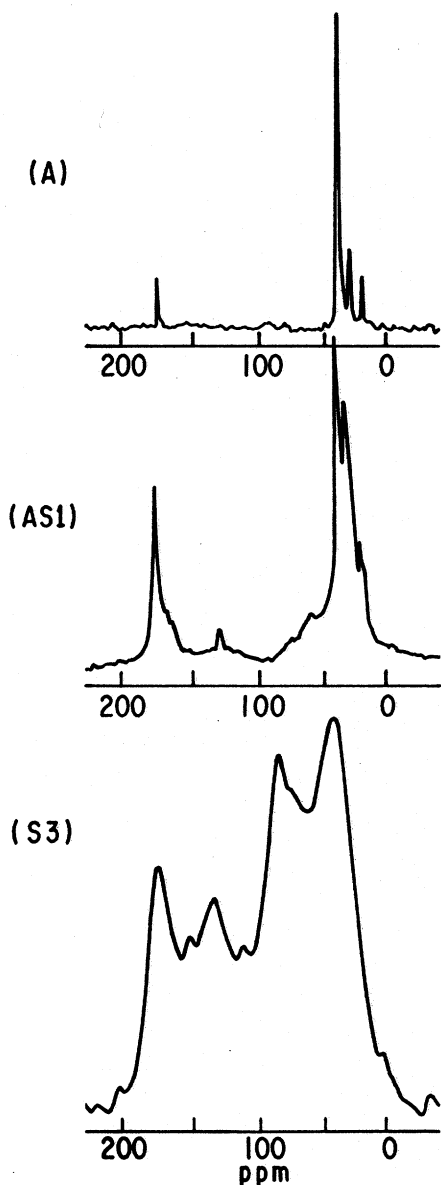
FIG. 4. CP-MAS ^{13}C NMR spectrum of tannery waste sludge (AS1) compared to the spectrum of stearic acid (A) and sewage sludge (S3).

erence compounds; these four compounds do give an approximation of the carbon types found in sludge spectrum S3. This simulated spectrum is not presented as representative of the chemical composition of sewage sludge, but rather as a means to profile sludge carbon functionalities.

Figure 6 contains the interrupted decoupling spectra of the four reference compounds. If these spectra are compared with the normal spectra in Fig. 1, many differences become apparent. In

between the two spectra. The peaks in the simulated spectrum are narrower with deeper valleys than the sludge spectrum. These four components cannot account for the dispersion of chemical shifts found in a complex mixture such as sludge, however, because these spectra of sludges are composed of broad envelopes of four carbon types, which are represented by the ref-
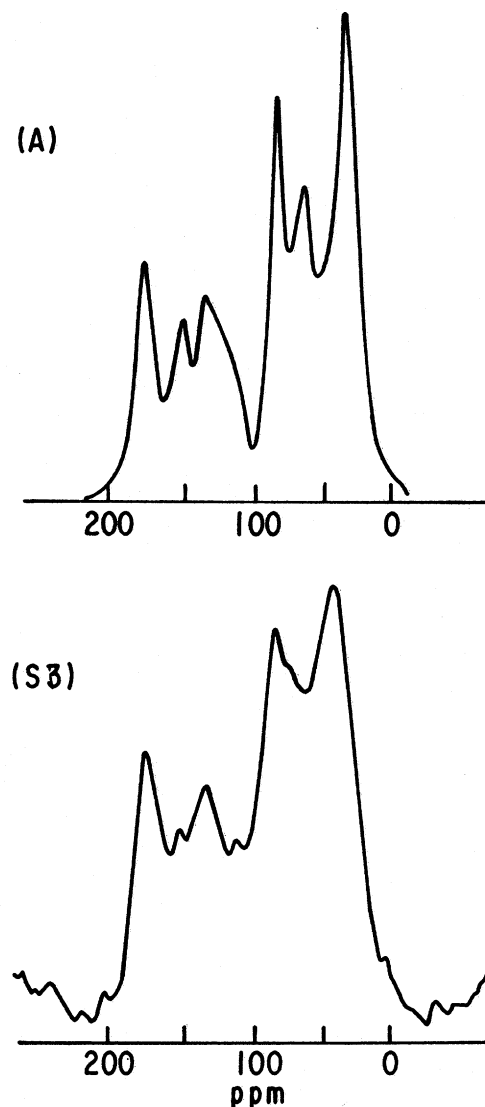

FIG. 5. (A) A computer-simulated spectrum of the four reference compounds composed of 25% stearic acid, 10% lignin, 30% protein (keratin), and 35% wood pulp compared to sewage sludge S3.
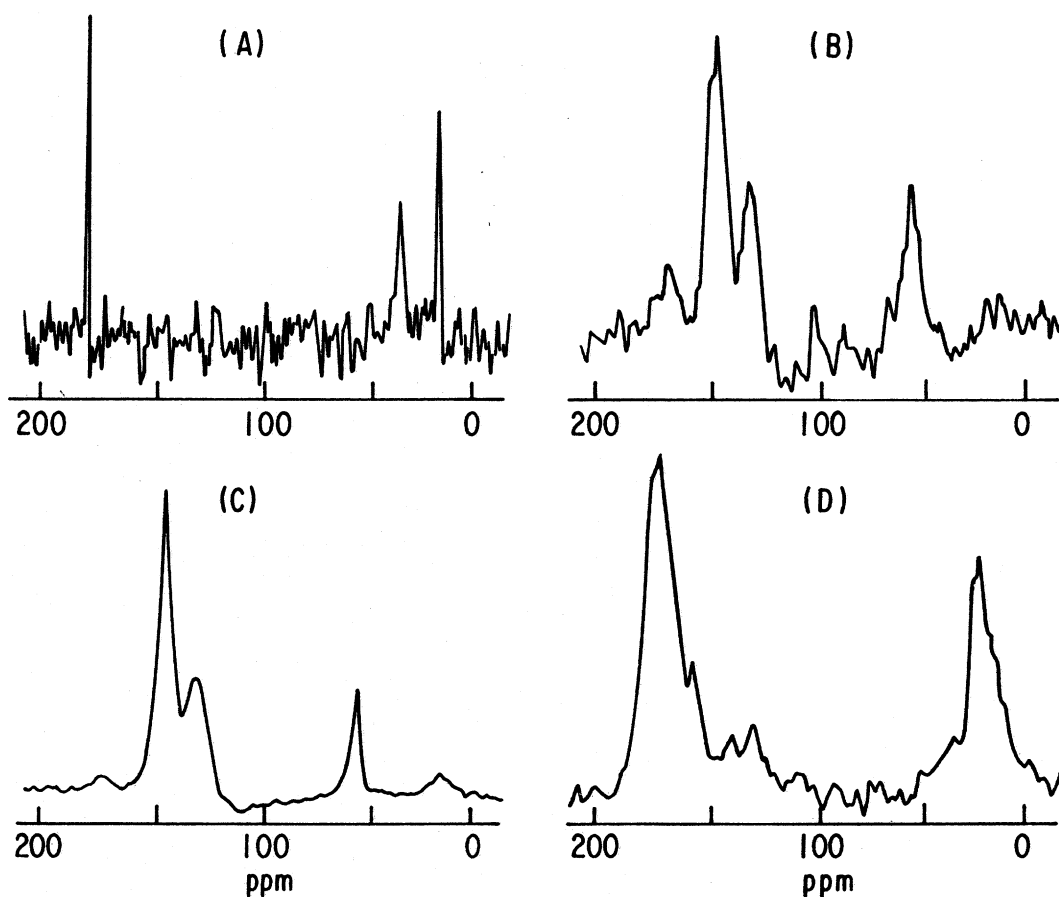
FIG. 6. CP-MAS NMR interrupted decoupling spectra (50-μs delay) of (A) stearic acid, (B) cellulose (wood pulp), (C) lignin, and (D) protein (keratin).

the interrupted decoupling experiment, the terminal methyl carbon of stearic acid (A) at 15 ppm gives a less intense response, due to residual proton broadening, than the carbonyl carbon in stearic acid (A) at 181 ppm. None of the cellulose carbons of spectrum (B) gives a response under the conditions of the experiment, because they undergo dipolar broadening with their associated protons. The only resonances observed in the interrupted decoupling spectra of wood pulp were those associated with lignin. The interrupted decoupling spectrum of lignin (C) gives a less intense response for the phenyl methoxy carbons at 55 ppm compared with the methoxy-substituted or hydroxy-substituted phenyl carbons at 148 ppm than the standard spectrum. The interrupted decoupling conditions produced a spectrum of keratin (D) that has a reduced intensity of aliphatic carbons relative to the

carbonyl, especially those bound to N. Free methyl is the major component of the aliphatic peak in this keratin spectrum. Most of the aromatic carbon signals have been broadened, and the carbonyl peak has become the prominent peak in the spectrum.

The spectra presented in Fig. 7 illustrate how an interrupted decoupling experiment can be used to differentiate between different components in a complex mixture. The lignin in wood pulp is difficult to detect in the standard spectrum (C). Under the conditions of the selective broadening experiment, all the cellulose resonances in the wood pulp (B) are broadened, and the presence of lignin becomes readily apparent when compared with the selectively broadened spectrum of lignin (A). This experiment could be useful for measuring lignin in cellulose preparations.
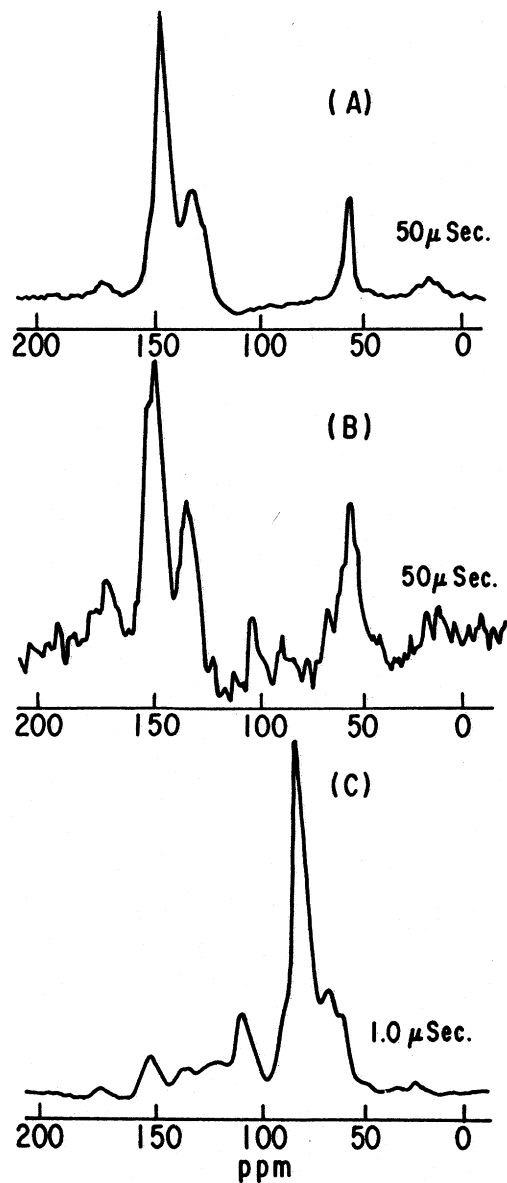
(A)

50 μ Sec.

200   150   100   50   0

(B)

50 μ Sec.

200   150   100   50   0

(C)

1.0 μ Sec.

200   150   100   50   0
ppm

FIG. 7. CP-MAS NMR interrupted decoupling spectra (50-μs delay) of lignin (A) and wood pulp (B) compared with the standard spectrum of wood pulp (C).

The interrupted decoupling spectrum of sludge S3 (B), presented in Fig. 8, can be anticipated after studying the interrupted decoupling spectra of the reference compounds. The carbohydrate resonances have undergone dipolar broadening as have the resonances due to C bound to N. Actually, the interrupted decoupling spectrum of sludge S3 looks similar to that of

keratin (A). The aliphatic peak in the interrupted decoupling spectrum of sludge S3 is larger than the aliphatic peak in the interrupted decoupling spectrum of the protein reference
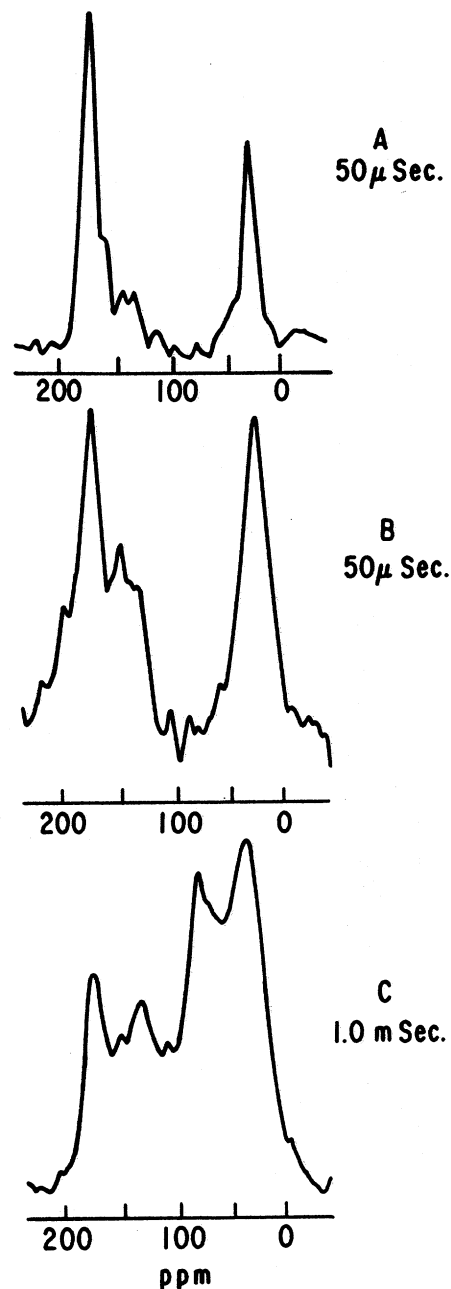
A
50 μ Sec.

200   100   0

B
50 μ Sec.

200   100   0

C
1.0 m Sec.

200   100   0
ppm

FIG. 8. CP-MAS NMR interrupted decoupling spectra (50-μs delay) of keratin (A) and sludge S3 (B) compared with a standard spectrum of sludge S3 (C).

when both are compared with the carbonyl peaks. This difference may be due to nonprotonated (branched) aliphatic carbons present in sludge S3, which are not found in keratin. The intensity of the aromatic carbons is greater in the sludge spectrum than the protein reference. If these aromatic shifts were due to lignin only, there would be two large sharp resonances at 152 and 57 ppm, corresponding to hydroxy or ether-linked aromatic and OCH_3 carbons, respectively. Sludge S3 does not contain enough lignin to account for all the aromatic intensity based on the lack of OCH_3 intensity at 57 ppm. Sludge S3 must contain other branched aromatic carbons. This additional nonprotonated aromatic and aliphatic carbon signal might be evidence of humic substances that are known to be present in sludges.

CONCLUSIONS

Solid-state ^{13}C CP-MAS NMR spectroscopy is an effective method for profiling the organic content of sewage sludges. Using the instrumental parameters established for this study, one can obtain spectra that show differences between sludges produced from varied sources by varied processes. A minimum of sample preparation is required to obtain these solid-state spectra. The complex sludge spectra provide general information about four carbon types: carbonyl, aromatic, carbon attached to –OH and –N, and aliphatic. The spectra indicate these sludges contain a large portion of carbon that is substituted with functional groups.

This solid-state technique is also applicable to sludge composts. The solid-state spectra indicate that the Beltsville composting procedure alters the organic composition of sewage sludges. There are significant differences between the spectra of the sludges and the composts in the regions of the carbonyl carbon, the carbon attached to –OH and –N, and the aliphatic carbon.

The interrupted decoupling experiments illustrate the effectiveness of the technique for enhancing the signal of nonprotonated (branched) systems by selectively broadening the signal of the protonated carbons in the sample. Line broadening simplified the spectrum of sludge S3 and indicated the presence of nonprotonated (branched) aliphatic and aromatic carbon. This branched aromatic component does not seem to be due to lignin.

Studying complex systems, such as sludge, by solid-state ^{13}C CP-MAS NMR is in the preliminary stages of development. More work is needed to understand how the numerous components, both organic and inorganic, in complex natural systems interact and how these interactions affect the chemical shifts and relaxation processes observed in the solid state.

Studies are currently being conducted to determine some of the ways metal ions affect the chemical shifts and the relaxation times of the organic components in sewage sludges. These studies will expand our understanding of the information that can be obtained about complex systems, such as sewage sludge, using solid-state ^{13}C CP-MAS NMR spectroscopy.

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